ORIGINAL ARTICLE

Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions

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Objective: To evaluate the effect of caffeine on white cell distribution and muscle injury markers in professional soccer players during exercise.
Methods: 22 male athletes completed a placebo controlled double blind test protocol to simulate a soccer match, followed by a Yo-Yo intermittent recovery test.
Results: Exercise caused an increase in packed cell volume that was enhanced by caffeine. Caffeine and exercise had a synergistic effect on the blood lymphocyte count, which increased by about 38% after exercise, and by an additional 35% when combined with caffeine. Caffeine promoted an exercise independent rise in circulating monocytes, and a synergistic action of exercise and caffeine was observed on segmented neutrophils. Caffeine promoted thrombocytosis. Plasma adenosine deaminase, aspartate aminotransferase, and lactate dehydrogenase concentrations were enhanced by exercise, and alanine transaminase concentration was enhanced in both groups, with a synergistic effect of caffeine.
Conclusions: The pronounced increase in the white cell count in the group receiving caffeine appeared to be caused by greater muscle stress and consequently more intense endothelial and muscle cell injury. The use of caffeine may augment the risk of muscle damage in athletes.

Several studies have found encouraging ergogenic effects of caffeine (1,3,7-trimethylxanthine) during endurance exercises by acting as a fatigue delayer and as an enhancer of the contractile strength of cardiac and skeletal muscle.[1 2] Nevertheless, adverse effects may occur in athletes who are susceptible to xanthine because of hormonal and metabolic features.[3 4]

Caffeine is an antagonist of adenosine A_1 and A_2 receptors and is also a psychomotor stimulant that quickly penetrates through the blood–brain barrier, increasing performance and delaying fatigue.[5] It is known that xanthine acts on the central nervous system (CNS) and on various different metabolic pathways, with the overall result that glycogen is conserved because of an increase in fat oxidation and a decrease in carbohydrate oxidation.[3] Distinct effects of caffeine are caused directly by adenosine receptor antagonism or indirectly by an increase in plasma adrenaline.[6] Caffeine changes the immune response, inducing leucocytosis, lymphocytosis, and neutrophilia along with an increase in metabolic rate.[7] Its use together with exercise activates both the hypothalamic-pituitary-adrenal axis and the autonomic nervous system, stimulating fast β-endorphin and cortisol release.[8] In addition, caffeine decreases muscular pain perception, effort perception, and the reaction time to a stimulus.[9]

Exercise causes an increase in some white blood cells such lymphocytes and neutrophils, leading to a mild leucocytosis.[10–12] Synergy between vascular endothelial growth factor (VEGF) released after vascular muscle damage and the products of cellular metabolism, especially ADP, stimulates mononuclear cells such as white cells to migrate to the circulatory system.[13] The strong muscle contractions during exercise may also cause micro-tears both in muscle and in the vascular endothelium, which also affect the migration of white cells.[14] In addition, many enzymes are released during skeletal muscle cell damage and exert immunomodulatory actions, serving as messengers to the immune system, in addition to being involved in circulating and tissue-bound leucocytes, cytokines, hormones, and growth factors causing muscle regeneration.[15 16]

Caffeine and caffeine based substances have been increasingly used as ergogenic supplements by athletes and soccer players, but its effects on the human immune system during physical exercise are still obscure. In the present study, we evaluated the effect of caffeine supplementation on the white cell count and muscle injury markers in soccer players during exercise.

METHODS

Subjects

Professional soccer players (n = 22) from a first division team affiliated to the Confederação Brasileira de Futebol (CBF, Brazilian Soccer Confederation) participated in this study voluntarily. These athletes had no medical history of health problems and were not using ergogenic substances or any other drugs. The team was in contention for the Brazilian Soccer Championship, which guaranteed that all the players had similar diets, training regimens, and resting and sleep conditions. This was essential to ensure the control of many experimental variables that could otherwise affect the results.

Clinical examinations, anthropometric measurements, and laboratory tests were carried out on the subjects to assure integrity and homogeneity among the three experimental groups (table 1). The initial laboratory evaluation tests included haematological and biochemical analyses, which allowed us to identify alterations in metabolism that could affect the results or impair their interpretation.

Abbreviations: C, caffeine only group; CEx, caffeine plus exercise group; LEx, lactose plus exercise group; VDR, variable distance run protocol; VEGF, vascular endothelial growth factor

Table 1 Age and anthropometric measurements in soccer players assigned to groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine)

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	Mean (SEM)	Range	Mean (SEM)	Range	Mean (SEM)	Range
Age (years)	26.0 (1.6)	19.9 to 33.2	25.3 (2.0)	19.0 to 37.3	31.0 (4.9)	24.3 to 40.5
Weight (kg)	77.5 (3.1)	64.3 to 92.4	76.1 (2.8)	65.4 to 86.1	71.6 (1.9)	68.3 to 74.9
Height (cm)	178 (2)	168 to 186	177 (3)	163 to 187	173 (2)	170 to 175

Values are mean (SEM) and range. No statistically significant differences were detected ($p>0.05$).

Experimental protocol

The study was double blind and randomised. It was approved by the ethics committee for human research from the University Castelo Branco and met the requirements for carrying out research on human subjects (Health National Council, Brazil, 1996). Written informed consent was obtained from the subjects, who were instructed as to the nature and procedures of the study.

Prior to training (D_0), blood and urine were collected from the fasting subjects. A clinical examination was done and anthropometric measurements made. No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 72 hours before blood collection.

On the 13th day of training (D_{13}), blood was collected from fasting soccer players (before breakfast (PRE)). The players were randomly divided into two groups and received a specific breakfast diet and a physical exercise programme: CEx = caffeine and exercise; LEx = lactose and exercise. Three athletes who were avoiding exercise for non-physical technical reasons were used as control (C = caffeine without exercise). We present these data but do not discuss them because of the small number of subjects.

After receiving breakfast and the supplements, the subjects were driven to the test place, which took 15 minutes. After 20 minutes of warming up (articular mobilisation and elongation exercises), the subjects undertook the test protocol under cardiac monitoring, simulating a soccer match (fig 1).

Specific procedures

Diet supplementation

The different supplements were in indistinguishable capsules so that the subjects were not aware of the substance they were ingesting. Caffeine (Purifarma, China) was given to groups CEx and C at a dose of 5 $mg.kg^{-1}$ in two 500 mg capsules, which were then completed with lactose (Via Farma, Brazil). The control group, LEx, received two capsules with 500 mg lactose each.

Test protocol

The variable distance run protocol (VDR) was used to simulate a soccer match and was carried out for 45 minutes in a 50×50 m court with 5×5 m marks. After hearing a beep, the athletes started running at top speed and in any direction. They randomly ran any of the 66 distances determined by a voice command following the beep and were separated by resting periods (frequency × distance + resting time: 7×10 m + 15 s; 12×20 m + 25 s; 15×30 m + 35 s; 17×40 m + 45 s, and 15×50 m + 60 s). At the end, all the athletes had run the same distances at variable speeds, which were correlated with each subject and associated with his soccer position. The athletes were allowed to drink a solution of electrolytes and glucose (Gatorade®) ad libitum throughout their training. After the VDR, a Yo-Yo intermittent recovery test (Yo-Yo IRT)[17] was carried out to drive the athletes to exhaustion. This test ended at different times for each athlete, and immediately afterwards blood was collected for laboratory analyses using a double blind procedure (POST).

Data collection

Preliminary analyses (D_0)

The preliminary tests were carried out on D_0 to ensure that the individuals taking part in the study were in good metabolic condition. Blood and urine were collected from fasting athletes

Table 2 Haematological variables in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine), determined on day 0

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	Mean (SEM)	Range	Mean (SEM)	Range	Mean (SEM)	Range
Erythrocytes ($\times10^{12}$/l)	5.3 (0.1)	5.0 to 5.4	5.0 (0.1)	4.7 to 5.2	4.7 (0.1)	4.4 to 4.9
Haemoglobin (mmol/l)	2.5 (0.1)	2.3 to 2.6	2.4 (0.1)	2.3 to 2.5	2.4 (0.1)	2.4 to 2.5
PCV (%)	47.3 (0.9)	44.3 to 49.5	45.9 (0.7)	43.5 to 47.9	45.3 (0.6)	44.3 to 46.8
MCV (fl)	89.3 (0.9)	85.7 to 92.0	91.3 (0.6)	89.0 to 92.6	95.7 (2.3)	91.0 to 100.7
MCH (pg)	30.1 (0.4)	28.8 to 31.2	30.8 (0.3)	29.9 to 31.9	32.6 (1.0)	30.6 to 34.8
MCHC (%)	33.7 (0.1)	33.2 to 34.1	33.7 (0.2)	33.1 to 34.5	34.0 (0.2)	33.6 to 34.5
Platelets ($\times10^3$)	218.8 (16.0)	175.0 to 281.0	193.7 (9.7)	155.0 to 221.0	240.3 (18.2)	206.0 to 282.0
Leucocytes ($\times10^9$/l)	6.72 (0.6)	5.0 to 8.4	6.2 (0.5)	4.2 to 8.0	5.7 (0.8)	4.5 to 7.7
Basophils	41 (14)	0 to 83	27 (10)	0 to 66	57 (8)	45 to 77
Eosinophils	148 (35)	60 to 252	251 (67)	51 to 560	192 (82)	45 to 385
Myelocytes	0 (0)	0 to 0	0 (0)	0 to 0	0 (0)	0 to 0
Metamyelocytes	0 (0)	0 to 0	0 (0)	0 to 0	0 (0)	0 to 0
Bands	67 (6)	50 to 84	92 (17)	32 to 160	73 (13)	45 to 98
Segmented	3613 (444)	2444 to 4884	2706 (464)	736 to 4118	2628 (304)	2025 to 3311
Lymphocytes	2391 (206)	1776 to 3108	2304 (151)	1764 to 2800	2325 (378)	1715 to 3234
Monocytes	456 (61)	250 to 592	376 (35)	256 to 497	425 (78)	315 to 616

Values are mean (SEM) and range.
No statistically significant differences were found among the designated groups.
n, number of athletes; PCV, packed cell volume.

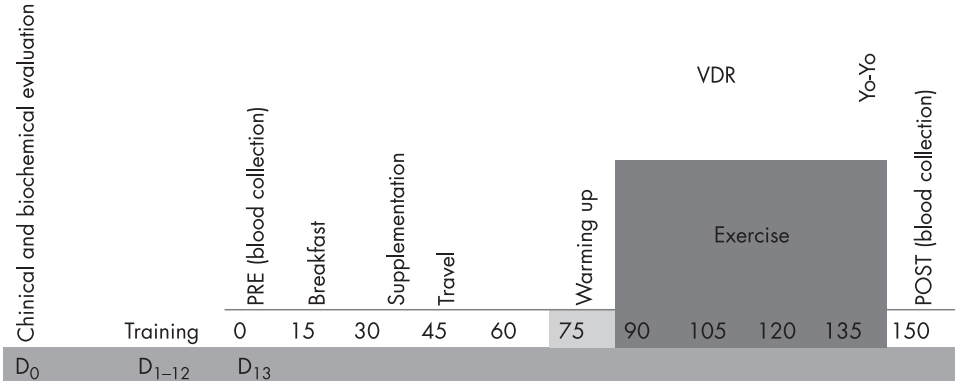

Figure 1 Experimental design and temporal curve. Before training, the athletes were submitted to a clinical and biochemical evaluation (D_0) as described in Methods. After 12 normal training days, blood was collected (PRE), and the athletes received food and caffeine; 60 minutes after supplementation, an exercise protocol was carried out, followed by another blood collection (POST). VDR, variable distance run protocol.

on the morning of the first day of training. Clinical examinations and anthropometric evaluations were done at the same time (tables 1–6).

Blood sampling (D_0 and D_{13})

Venous blood samples were collected from the forearm into heparinised tubes. Immediately after the collection, the blood samples were centrifuged for plasma separation. Plasma was quickly frozen and stored at −70°C. The samples were analysed by Laboratório Bittar Ltda (Niterói, Brazil).

A range of analyses was carried out to detect any variable that could affect the results; urinary myoglobin and troponin I were also determined for evaluation of muscle integrity (data not shown).

Statistical analyses

Student's *t* test or analysis of variance was used to compare the treatments, and significant differences were set at $p<0.05$. Data are expressed as mean (SEM).

RESULTS

Preliminary tests (D_0)

Unless stated, all the baseline haematological and biochemical variables were within the normal range for the group.

Athletes from all the groups had normal red and white cell counts at D_0 (table 2). The results from all the other analyses were also normal, and no difference was found within or among the groups. Ketone bodies and lipid profiles were similar among the groups, and the high density lipoprotein (HDL) concentration was high in all groups (mean (SEM), mmol/l: CEx = 55.2 (2.8); LEx = 52.9 (2.8), C = 53.7 (3.8)). Although cholesterol concentrations were higher in CEx (199.5 (6.2) mmol/l) than in C (150.0 (16.9)), this difference was not considered further because the main comparison was between CEx and LEx.

Serum biochemical analyses and serum hormones were within the normal population range. The plasma sodium concentration was slightly lower in CEx than in the other groups (tables 3 and 4). Serum concentrations of creatine kinase (CK) and creatinine kinase MB isoform (CKMB) were above normal levels in 14 subjects (table 5), and troponin I and urinary myoglobin were at normal levels for all athletes. C reactive protein concentration was also within the normal values for all the groups (data not shown).

Exercise test (D_{13})

The haematological profile was affected by exercise, with an additional effect of caffeine. Erythrocyte count, haemoglobin,

Table 3 Biochemical variables in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 0

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**
Glucose (mmol/l)	5.0 (0.1)	4.8 to 5.1	5.0 (0.1)	4.4 to 5.2	4.9 (0.3)	4.5 to 5.4
Lactate (mmol/l)	4.3 (0.4)	2.6 to 5.4	4.6 (0.5)	2.8 to 6.2	4.8 (0.1)	4.6 to 4.9
Ammonia (μmol/l)	63.1 (13.5)	24 to 137	50.7 (5.9)	28 to 69	80.0 (33.5)	37 to 146
Urate (μmol/l)	350.7 (11.6)	308.6 to 390.8	340.6 (33.7)	233.1 to 438.8	388.5 (41.2)	308.6 to 445.7
Creatinine (μmol/l)	89.7 (3.6)	70.7 to 97.2	91.3 (3.4)	79.6 to 106.1	94.3 (2.3)	88.4 to 97.2
Urea (mmol/l)	4.6 (0.5)	3.2 to 6.8	5.6 (0.4)	4.2 to 7.2	5.6 (1.1)	3.5 to 7.0
Chloride (mmol/l)	100.0 (0.6)	98.1 to 102.1	100.6 (0.8)	98.1 to 104.1	99.0 (0.1)	99.1 to 99.3
Phosphorus (mmol/l)	4.0 (0.3)	3.2 to 5.3	3.4 (0.2)	2.7 to 4.7	3.5 (0.2)	3.1 to 3.8
Potassium (mmol/l)	4.3 (0.2)	3.8 to 5.1	4.3 (0.2)	3.8 to 5.0	4.2 (0.3)	3.7 to 4.5
Sodium (mmol/l)	141.5 (0.7)	140 to 144*	144.1 (0.8)	141 to 147*	144.0 (1.5)	141 to 146
Total protein (g/l)	78.2 (1.8)	73.7 to 82.8	77.0 (0.5)	74.7 to 78.7	76.1 (3.5)	70.8 to 82.8
Albumin (g/l)	56.0 (4.3)	48.5 to 83.7	50.8 (1.4)	46.9 to 58.0	51.7 (3.1)	46.4 to 57.0
α_1 Globulin (g/l)	1.2 (0.1)	0.8 to 1.6	1.3 (0.2)	1.0 to 2.3	1.4 (0.1)	1.3 to 1.5
α_2 Globulin (g/l)	5.5 (0.4)	4.4 to 7.5	4.4 (0.6)	2.6 to 7.2	4.8 (0.8)	3.3 to 5.8
β Globulin (g/l)	7.6 (0.3)	6.6 to 8.6	7.1 (0.5)	5.1 to 8.6	6.8 (0.3)	6.2 to 7.4
γ Globulin (g/l)	11.8 (0.6)	9.7 to 14.4	13.5 (1.1)	10.4 to 19.6	11.4 (0.8)	9.7 to 12.2
A/G ratio	2.0 (0.1)	1.8 to 2.3	2.0 (0.2)	1.6 to 2.9	2.1 (1.1)	1.9 to 2.2
Bilirubin, direct (μmol/l)	6.5 (0.1)	5.1 to 10.2	5.6 (0.1)	5.1 to 6.8	6.0 (1.0)	4.1 to 7.0
Bilirubin, indirect (μmol/l)	7.5 (0.1)	5.1 to 15.4	6.0 (0.1)	3.4 to 8.5	7.2 (0.7)	6.5 to 8.7
Bilirubin, total (μmol/l)	14.0 (0.1)	10.3 to 25.6	11.6 (0.1)	8.5 to 15.4	13.3 (1.5)	10.6 to 15.7

Values are mean (SEM) and range.
*Significant difference in concentrations between CEx and LEx ($p<0.05$).
A/G, albumin/globulin ratio; n, number of athletes.

Table 4 Hormonal levels in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 0

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**
Dopamine (pmol/l)	7.8 (0.7)	5.4 to 10.3	10.4 (1.4)	5.0 to 14.8	8.7 (2.4)	5.1 to 13.3
Adrenaline (pmol/l)	86.3 (9.5)	51.6 to 115.7	62.5 (9.7)	41.8 to 113.7	103.6 (8.9)	89.0 to 119.6
Noradrenaline (nmol/l)	362.3 (108.0)	187.8 to 1000.1	250.1 (33.2)	121.7 to 369.0	222.5 (39.0)	152.0 to 286.5
TSH (μU/l)	2.3 (0.3)	1.3 to 3.6	2.6 (0.6)	1.1 to 5.6	2.0 (0.7)	1.1 to 3.4
T_3 (nmol/l)	2.4 (0.1)	2.4 to 2.5	2.4 (0.1)	2.4 to 2.5	2.4 (0.1)	2.4 to 2.5
FT_3 (nmol/l)	55.5 (3.0)	43.1 to 63.1	59.2 (1.9)	49.3 to 63.4	56.8 (6.1)	44.7 to 63.1
T_4 (nmol/l)	90.6 (3.7)	77.3 to 108.4	91.3 (5.5)	69.8 to 112.7	97.3 (5.8)	86.9 to 106.9
FT_4 (pmol/l)	12.4 (0.4)	11.0 to 14.3	12.6 (0.6)	9.5 to 14.4	13.5 (0.4)	12.7 to 13.3
Testosterone (nmol/l)	30.1 (3.0)	16.9 to 39.9	34.7 (3.5)	24.2 to 51.4	24.0 (2.9)	19.6 to 29.5
Free testosterone (pmol/l)	36.3 (1.1)	31.6 to 38.9	35.9 (0.8)	32.2 to 38.6	35.5 (0.6)	34.9 to 36.7
Basal GH (μg/l)	0.4 (0.1)	0.1 to 0.8	0.2 (0.1)	0.1 to 0.4	0.3 (0.2)	0.1 to 0.6
Insulin (μU/l)	5.2 (0.2)*	4.7 to 6.1	6.2 (0.4)*	4.6 to 7.6	5.3 (0.4)	4.8 to 6.2

Values are mean (SEM) and range.
No significant differences were found among the subjects or groups, except for blood insulin level.
*$p<0.05$, CEx *v* LEx.
FT_3, free tri-iodothyronine; FT_4, free thyroxine; GH, growth hormone; n, number of athletes; TSH, thyroid stimulating hormone.

Table 5 Enzyme levels in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 0

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**	**Mean (SEM)**	**Range**
ADA (U/l)	23.8 (1.3)	18.3 to 27.6	22.4 (1.2)	16.2 to 24.8	23.5 (2.3)	20.1 to 27.8
AP (U/l)	67.7 (5.8)	51.1 to 91.0	58.4 (2.5)	52.0 to 71.1	59.0 (3.6)	54.1 to 66.1
ALT (U/l)	29.0 (3.9)	16.0 to 40.1	27.3 (3.6)	15.1 to 39.0	26.7 (2.8)	21.1 to 30.0
AST (U/l)	27.4 (2.3)	19.1 to 36.0	27.3 (2.7)	19.1 to 40.1	25.0 (1.2)	23.1 to 27.0
CK (U/l)	221.2 (47.5)	109.1 to 467.0	264.1 (65.4)	75.2 to 569.1	139.3 (25.8)	97.1 to 186.1
CKMB (U/l)	155.4 (53.1)	16.5 to 356.6	184.0 (60.5)	15.3 to 468.5	43.1 (12.7)	18 to 59.6
LDH (U/l)	247.3 (9.8)	218.1 to 287.2	260.3 (18.3)	193.1 to 336.2	255.7 (8.0)	244.0 to 271.1

Values are mean (SEM) and range.
No statistically significant differences were found among the groups.
ADA, adenosine deaminase; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatinine kinase; CKMB, creatinine kinase MB isoform; LDH, lactate dehydrogenase; n, number of athletes.

Table 6 Haematological variables in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 13

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variables	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
Erythrocytes ($\times 10^{12}$/l)	5.1 (0.1)	5.3 (0.1)*§	4.8 (0.1)	4.9 (0.1)*§	4.6 (0.1)	4.7 (0.2)
Haemoglobin (mmol/l)	2.4 (0.1)	2.5 (0.1)*	2.3 (0.1)	2.4 (0.0)	2.4 (0.1)	2.4 (0.2)
PCV (%)	45.9 (1.1)	47.6 (1.0)*§	45.3 (0.7)	45.8 (1.0)§	45.3 (1.0)	45.5 (1.2)
MCV (fl)	90.2 (0.9)	90.2 (1.1)§	93.5 (0.4)	93.5 (0.5)§	98.5 (2.9)	97.5 (2.5)
MCH (pg)	30.1 (0.4)	29.8 (0.4)	31.0 (0.2)	30.9 (0.2)	32.7 (1.3)	32.4 (1.3)
MCHC (%)	33.4 (0.2)	33.0 (0.2)	33.1 (0.1)	32.9 (0.1)	33.6 (0. 3)	33.2 (0.5)
Platelets ($\times 10^3$)	223.9 (11.3)	276.9 (13.4)*†§	202.4 (10.2)	225.2 (12.1)*‡§	237.0 (31.8)	247.3 (28.6)†‡
Leucocytes ($\times 10^9$/l)	6.4 (0.4)	10.3 (0.8)*†§	5.3 (0.5)	7.1 (0.9)*‡§	6.7 (1.1)	7.0 (1.2)†‡
Basophils	34.4 (12)	63 (19)	22 (9)	15 (9)	50 (27)	18 (18)
Eosinophils	157 (20)	152 (23)	237 (36)	250 (52)	138 (21)	140 (30)
Myelocytes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Metamyelocytes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bands	82 (9)	133 (16)*	90 (15)	136 (20)	84 (15)	107 (3)
Segments	3465 (304)	5482 (617)*†§	2607 (346)	3423 (567)*§	3787 (1194)	4118 (1341)*†
Lymphocytes	2126 (97)	3671 (317)*†	1977 (139)	2740 (358)*	2234 (182)	2087 (124)†
Monocytes	535 (58)	779 (64)*	403 (41)	498 (84)	407 (51)	530 (47)*

Values are mean (SEM).
*$p<0.05$, PRE *v* POST.
†$p<0.05$, C POST − C PRE *v* CEx POST − CEx PRE (ΔC *v* ΔCEx).
‡$p<0.05$, ΔC *v* ΔLEx.
§$p<0.05$, ΔLEx *v* ΔCEx.
n, number of athletes; PCV, packed cell volume.

Table 7 Plasma protein concentrations in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 13

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variables	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
Total protein (g/l)	72.7 (0.8)	80.7 (1.2)*§	71.9 (0.6)	75.7 (0.7)*§	71.6 (2.8)	75.8 (3.0)
Albumin (g/l)	48.3 (0.7)	50.8 (1.4)	48.0 (0.9)	48.0 (0.9)	47.9 (1.4)	51.3 (0.9)
α_1 globulin (g/l)	1.3 (0.1)	1.7 (0.3)	1.3 (0.1)	1.2 (0.1)	1.3 (0.1)	1.3 (0.2)
α_2 globulin (g/l)	4.4 (0.4)	5.2 (0.4)	4.1 (0.4)	4.2 (0.4)	3.7 (0.8)	3.8 (0.9)
β globulin (g/l)	7.2 (0.4)	9.2 (0.5)*	6.9 (0.3)	8.5±0.3*	6.1 (0.6)	7.2 (1.0)
γ globulin (g/l)	11.4 (0.6)	13.4 (0.8)*	11.7 (0.5)	13.7 (0.5)*	12.6 (1.6)	12.2 (2.0)
A/G ratio	2.0 (0.1)	1.7 (0.1)*	2.0 (0.1)	1.7 (0.1)	20.9 (3.0)	21.7 (2.9)

Values are mean (SEM).
*p<0.05, PRE *v* POST; §p<0.05, ΔLEx *v* ΔCEx.
A/G, albumin/globulin ratio; n, number of athletes.

and packed cell volume values increased by about 4% in CEx (table 6), and the red cell count increased by nearly 2% in LEx.

The white blood cell population was modified by both exercise and caffeine supplementation. The leucocyte count increased by 34% in LEx and by 61% in CEx, with an overall increase of nearly 80% in the supplemented group compared with LEx. Segmented neutrophils increased by 31% in LEx and by 58% in CEx. Lymphocytes increased 45% in LEx and 77% in CEx, and circulating monocytes increased by 30% in C and 50% in CEx. The thrombocyte count increased by 11% in LEx and by 24% in CEx (table 6).

After exercise, the total protein increased by 11% in CEx and by 5% in LEx, and this effect was significantly more pronounced in the CEx group. Both β and γ globulins were increased by exercise, independent of caffeine, and the effect was greater for β globulins (table 7).

For plasma enzyme determinations, no differences were found for γGT (table 8), troponin I, or plasma myoglobin (data not shown) between PRE and POST periods or among the groups. Exercise caused an increase of 34% in plasma lactate dehydrogenase (LDH) concentration in the CEx group, similar to the unsupplemented LEx group. The same behaviour was observed for aspartate aminotransferase (AST) and adenosine deaminase (ADA) concentration, but the increase was less pronounced. The increase in alkaline phosphatase (AP) was 24% in the CEx group. Alanine aminotransferase (ALT) also rose significantly in both groups, the increase being approximately 50% greater in CEX than in LEx. There was no change in γ-glutamyl transferase (γGT) in any of the groups (table 8).

A rise in CK was observed between the initial and final levels in both exercise groups (CEx = 30%, LEx = 13%). We plotted the CK values as a function of ALT, AST, AP, and γGT to investigate the origin of the raised CK, but no concentration differences were found among these enzymes (figs 2–5). Figure 2 shows the enhancement in CK and ALT for the CEx and LEx groups, in both cases significantly different from C. An increase in AST and AP *v* CK was found only in CEx (figs 3 and 4), and no differences were found by linear regression analysis among CEx, LEx, and C. To analyse the origin of AST and ALT we plotted CK against γGT concentrations, but no significant differences were detected (fig 5).

DISCUSSION

Caffeine supplementation has been shown to enhance both performance and reaction time in athletes practising different physical activities.[2 18 19] In this study we examined the effects of caffeine supplementation on haematological variables, plasma proteins, and liver enzymes in soccer players under physical stress condition (a simulation of a soccer match). The adequacy of the experimental model was assured by the similarity among the subjects, all of whom were professional soccer players from a first division team, under an intense pre-game regimen and thus having the same training programmes, diet, and resting and sleeping hours.

Table 8 Changes in enzymes in the soccer players from groups CEx (caffeine and exercise), LEx (lactose and exercise) and C (caffeine) determined on day 13

	CEx (n = 11)		LEx (n = 8)		C (n = 3)	
Variable	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
ADA (U/l)	23.4 (0.7)	24.3 (0.5)*	22.3 (1.0)	23.6 (0.7)*	22.5 (3.0)	24.1 (3.5)*
AP (U/l)	69.6 (5.6)	86.4 (4.3)*	73.0 (6.2)	78.6 (8.1)	61.0 (1.5)	61.7 (3.9)†
ALT (U/l)	27.7 (2.1)	45.1 (3.3)*§†	31.7 (1.8)	44.6 (2.9)*§‡	33.7 (5.2)	27.7 (1.5)†‡
AST (U/l)	37.4 (3.4)	49.8 (4.9)*†	38.0 (3.3)	50.5 (5.0)*	48.3 (9.2)	32.7 (5.8)†
γ GT (U/l)	26.7 (1.7)	28.8 (2.1)	32.8 (6.0)	33.1 (6.9)	28.7 (2.0)	29.7 (3.8)
CK (U/l)	527.9 (129.3)	682.0 (172.3)*†§	665.3 (129.8)	750.6 (140.0)§	365.3 (173.7)	363.7 (163.8)†
CKMB (U/l)	17.3 (3.6)	25.6 (4.0)*	17.3 (2.4)	22.2 (3.2)	14.7 (5.1)	16.1 (5.8)
LDH (U/l)	293.0 (17.6)	392.2 (25.5)*†	327.4 (20.5)	412.5 (25.1)*‡	264.3 (31.7)	269.0 (36.7)†‡
Bilirubin, direct (μmol/l)	5.3 (0.0)	5.6 (0.1)	3.8 (0.0)	5.2 (0.1)*	4.3 (0.1)	4.4 (0.4)
Bilirubin, indirect (μmol/l)	8.2 (0.1)	9.3 (0.1)	7.2 (0.0)	8.1 (0.1)	7.0 (0.8)	7.7 (1.0)
Bilirubin, total (μmol/l)	12.2 (0.1)	14.9 (0.1)*	11.1 (0.1)	13.2 (0.1)*	11.3 (0.6)	12.1 (0.8)

Values are mean (SEM).
*p<0.05, PRE *v* POST; †p<0.05, ΔC *v* ΔCEx; ‡p<0.05, ΔC *v* Δ LEx; §p<0.05, ΔLEx *v* ΔCEx.
ADA, adenosine deaminase; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatinine kinase; CKMB, creatinine kinase MB isoform; γGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; n, number of athletes.

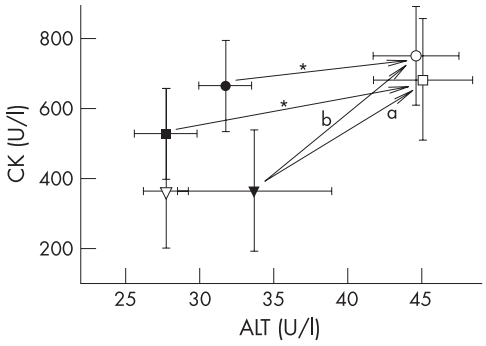

Figure 2 Correlation between creatinine kinase (CK) and alanine aminotransferase (ALT) measured before and after physical exercise. Filled square, CEx PRE; empty square, CEx POST; filled circle, LEx PRE; empty circle, LEx POST; filled triangle, C PRE; empty triangle, C POST. *$p<0.05$, PRE *v* POST; [a]$p<0.05$, ΔC *v* ΔCEx); [b] $p<0.05$, ΔC *v* Δ LEx.

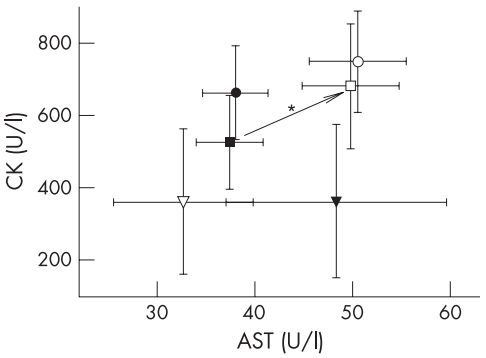

Figure 3 Correlation between creatinine kinase (CK) and aspartate aminotransferase (AST) measured before and after physical exercise. Filled square, CEx PRE; empty square, CEx POST; filled circle, LEx PRE; empty circle, LEx POST; filled triangle, C PRE; empty triangle, C POST. *$p<0.05$, PRE *v* POST.

Many studies on exercise metabolism have used apparently healthy subjects, but there have been no preliminary physiological analyses guaranteeing that these conditions held true. In our view, a preliminary evaluation is necessary for subject selection because metabolic disorders may lead to erroneous and controversial conclusions. In the present study, this assessment of the subjects assured their homogeneity as a group, not only in relation to clinical and anthropometric data, age, and sex, but also in metabolic terms. The 85 biochemical analyses undertaken allowed us to select individuals with similar carbohydrate, lipid, and protein metabolism, similar oxygen transport efficiency, similar macronutrient anabolism and catabolism indicators, normal water and electrolyte metabolism, no infection or parasitic infestation, a well balanced body water content; and undisturbed hepatic and renal function. Reinforcing the usefulness of pre-evaluation, one athlete was excluded because asymptomatic hepatitis was detected. In addition the extensive analyses gave us reference data for future evaluations.

Based on previous data on caffeine pharmacokinetics and dose dependency during both rest and physical exercise,[5 18] we used a 5 $mg.kg^{-1}$ caffeine dose in this study. This is within the positive supplementation range of 3–9 $mg.kg^{-1}$ body weight and thus allowed us to predict an improvement in the athletes' performance during the experiment, even though this was not the main focus of this study.

Endurance training may affect the red cell count and impair physical performance.[13 20] We found a significant exercise induced increase in packed cell volume enhanced by caffeine in our investigation. In addition we did not find any change in blood volume, which indicates that the enhancement was a result of red blood cell mobilisation by exercise.

Exercise increases the circulating red and white cell counts, largely because of their mobilisation from blood storage sites.[13] With respect to circulating leucocytes, we found a synergistic effect of caffeine in addition to exercise. The total white cell count in the blood increased almost twofold more in response to exercise than in the non-supplemented group. We showed a release of lymphocytes with exercise, and caffeine also promoted a rise in circulating monocytes. A synergistic action of exercise and caffeine (group CEx) was observed on segmented neutrophils. Exercise enhanced the lymphocyte count by about 38%, and when the effect of caffeine was added, the count increased by an additional 35%. These results are reinforced by the findings of Ramanaviciene *et al*,[7] who

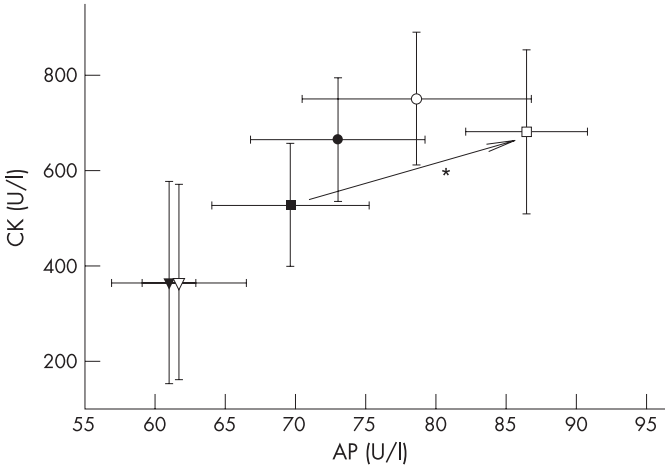

Figure 4 Correlation between creatinine kinase (CK) and alkaline phosphate (AP) measured before and after physical exercise. Filled square, CEx PRE; empty square, CEx POST; filled circle, LEx PRE; empty circle, LEx POST; filled triangle, C PRE; empty triangle, C POST. *$p<0.05$, PRE *v* POST.

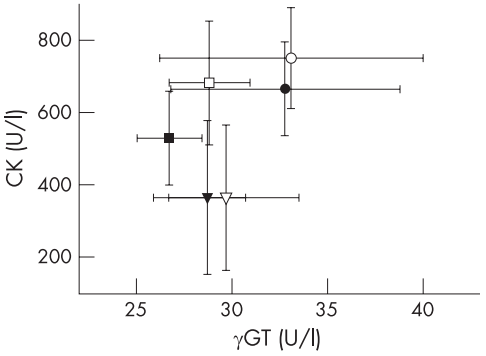

Figure 5 Correlation between creatinine kinase (CK) and γ-glutamyl transferase (γGT) measured before and after physical exercise. Filled square, CEx PRE; empty square, CEx POST; filled circle, LEx PRE; empty circle, LEx POST; filled triangle, C PRE; empty triangle, C POST. No statistically significant differences were detected.

What is already known on this topic

- Caffeine is a well described ergogenic aid. Xanthines act as fatigue delayers and muscle strength enhancers, acting on the central nervous system.
- Caffeine has several metabolic effects including a rise in fat oxidation and the maintenance of glycogen stores.
- These features have led to an increase in caffeine use in athletes.

What this study adds

- This study showed important effects of caffeine on the white blood cell count in exercising footballers, suggesting that it aggravates the effects of muscle injury.

showed that caffeine improved immunological activity by increasing the mobilisation of lymphocytes.

Exercise causes thrombocytosis, and as platelets respond to stimuli that recruit white cells from blood storage sites, they are possibly modulated by factors that affect leucocytosis.[11 21] Physical stress promotes cell damage, accompanied by an acute inflammatory process[22] which results in the mobilisation of lymphocyte subpopulations into the blood stream after exercise; thus the lymphocyte count increases during activity and declines once exercise ceases.[12] Platelets may have increased because of the muscle and vascular trauma that occurs during physical exercise. The additional effect of caffeine on platelets may reflect its action on purinergic receptors—a proinflammatory action that appears to be mediated by adenosine monophosphate and protein kinase, or to be caused by release from the spleen.[14]

We found an enhancement of plasma ADA, AST, and LDH in the exercise groups. ALT was also increased in both groups, with a synergistic effect of caffeine. The impact of exercise and caffeine on these enzymes could reflect a loss of hepatocyte or muscle cell membrane integrity with consequent leakage of the proteins into the blood. Concentrations of AP and γGT in the blood were not changed in any of the groups, in agreement with Haralambie's findings,[23] ruling out the possibility of hepatic injury. Thus the increase in plasma LDH, ALT, and AST suggests that the muscle lesions caused by exercise are enhanced by caffeine.[24 25]

The biggest increase in blood CK and LDH concentrations is detected in a 24–72 h post-exercise window.[26] In our study, blood was sampled immediately after physical exercise because of our interest in metabolic changes. This early collection explains why the concentrations of these enzymes were much lower here than in other studies.[25] The CK concentration increased more in the CEx group than in the LEx group, probably because CKMB increased significantly in the CEx group. These data indicate that the increases in the enzymes analysed in this study resulted from muscle injury, and that caffeine increased the exercise induced damage, in agreement with Hoffman *et al*.[24]

Our study suggests that the pronounced increase in the white cell count in CEx was caused by greater muscle stress and consequently more intense endothelial and muscle injury. The immune system may play a role in modulating skeletal muscle repair after exercise injury, so the degree of exercise induced muscle damage is reflected by the subsequent leucocytosis. The subacute inflammatory process in group CEx, coupled with catecholamine release, resulted in enhancement of ADA concentration. This could reflect a subacute inflammatory response associated with adrenaline release rather than an effect of caffeine, as caffeine acts directly on white cells.[26 27] Thus the increase in the white blood cell count in athletes from groups CEx and LEx was probably caused by mechanical effects rather than by hormonal action, in agreement with Boyum *et al*.[26] Overall, the use of caffeine appears likely to aggravate muscle injury in athletes and may be one of the causes for the observed changes in white cell response.

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COMMENTARY

This paper appears at a time when the World Anti-Doping Agency has decided to remove caffeine from the doping list, which has resulted in a great increase in the use of xanthine. The data presented here on the worldwide use of caffeine as an ergogenic aid are of great importance. The possibility of an increase in muscle stress should be considered for all potential users of caffeine in sport and physical activities.

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EDITORIAL BOARD MEMBER

Timothy Noakes

Timothy David Noakes, MB ChB, MD, FACSM, holds the Discovery Health Chair of Exercise and Sports Science at the University of Cape Town. He is also Director of the UCT/MRC Research Unit for Exercise Science and Sports Medicine and co-founder with Morné du Plessis of the Sports Science Institute of South Africa. While a student at UCT, he rowed for the South African Universities on two consecutive seasons and was awarded University full Blue colours. He began long distance running in 1972, and, at the time of his last marathon in 1990, had run more than 70 marathon and ultra-marathon races including seven Comrades Marathons (best time 6 h 49 min) and 15 Two Oceans marathons (best time 3h 59 min). In recent years he has been more active in cycling and triathlon events and has completed the Argus 105-km cycle race on eight occasions.

Among other things, he is working on the role of the brain in regulating exercise performance and fatigue during exercise and the role of fluid ingestion in aiding performance and reducing medical risks.

Noakes is the author or co-author of more than 250 scientific publications and is on the editorial boards of eight international scientific publications. He is a Fellow of the American College of Sports Medicine. In 1992, he was elected a Fellow of the University of Cape Town for sustained excellence in original scientific work. In 1992 and 1993, he was also one of the three finalists for the Burroughs Wellcome Gold Medal Award for Medical Research. In 1993, he received the Medical Research Council Publications Award for the best publication record in the previous three years by a South African medical scientist. In 1996, he was awarded the Citation Award of the American College of Sports Medicine and was the first South African to present the prestigious J B Wolffe Memorial Lecture at the American College of Sports Medicine. In 1999, he was elected as one of 22 founding members of the International Olympic Committee (IOC) Olympic Science Academy.

His book *Lore of running* is in its third edition in South Africa and the United States and has been translated for release in Japan. It is widely praised as the most complete such book yet written. Noakes is also co-author of *Running injuries* (with Steve Granger), *Lore of cycling*, *Running your best* (with Steve Granger) and *Rugby without risk* (with Morné du Plessis).

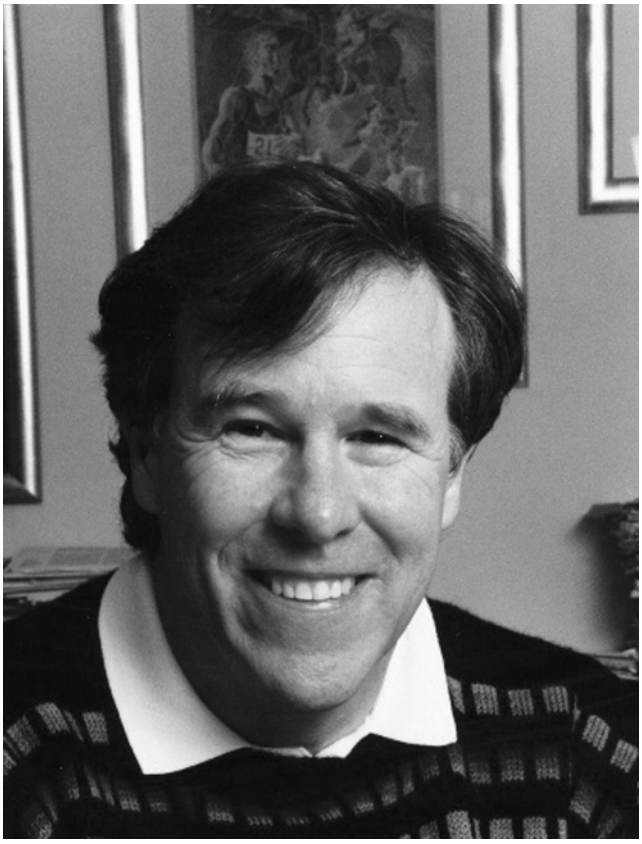

Figure 1 Timothy Noakes.

Noakes is married to Marilyn Anne. They have two children, Travis aged 28 and Candice aged 26.